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Engineering Optimized Scfv-fc Antibody-drug Conjugates (adcs) Targeting Galectin-3bp for Enhanced Cancer Therapy

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ADCs have revolutionized cancer treatment by selectively delivering cytotoxic agents to malignant cells while minimizing damage to healthy tissues. Galectin-3-Binding Protein (Gal-3BP) is a key target due to its role in immune evasion and metastasis. Based on the SP-2 antibody which has shown efficacy in vitro, we developed an ADC delivering mertansine (DM1). As the SP-2 epitope includes carbohydrate moieties that vary with the cellular environment, we engineered mutants that avoid carbohydrate binding to enhance specificity, therapeutic consistency, and stability.

In silico analyses, including molecular dynamics (MD) simulations, allowed to design modifications of the SP-2 scFv-Fc construct, which was then expressed in ExpiCHO cells and conjugated under an inert atmosphere using TCEP and DTNB reagents. High-temperature MD simulations revealed stable flexibility below 5 Å, validating the scFv-Fc epitope. Docking analyses identified mutants with enhanced stability, corroborated by further MD simulations. The native scFv-Fc exhibited satisfactory expression and Gal-3BP binding, but DM1 conjugation efficiency was suboptimal, likely due to purification and processing steps.

This study, integrating computational design, recombinant expression and chemical conjugation, offers a robust framework for optimizing ADCs, paving the way for improved targeted cancer therapies.